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Sinorhizobium morelense sp. nov., a *Leucaena leucocephala*-associated bacterium that is highly resistant to multiple antibiotics

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***Sinorhizobium morelense* sp. nov. is described to designate a group of bacteria isolated from root nodules of *Leucaena leucocephala*. *S. morelense* shows 98% 16S rRNA gene sequence similarity to some *Sinorhizobium* species and to *Ensifer adhaerens*. This novel species is distinguished from other *Sinorhizobium* species and from *E. adhaerens* by DNA–DNA hybridization, 16S rRNA gene restriction fragments and sequence and some distinctive phenotypic features. Strains of this species are highly resistant to some antibiotics, such as carbenicillin (1 mg ml^{-1}), kanamycin (500 μg ml^{-1}) and erythromycin (300 μg ml^{-1}). They do not form nodules, but a nodulating strain, Lc57, is closely related to the novel species. Strain Lc04^T (= LMG 21331^T = CFN E1007^T) is designated as the type strain of this novel species.**

Keywords: *Sinorhizobium morelense*, phylogeny, DNA–DNA relatedness, antibiotic resistance

INTRODUCTION

The soil bacteria within the genera *Azorhizobium* (Dreyfus *et al*., 1988), *Allorhizobium* (de Lajudie *et al*., 1998b), *Bradyrhizobium* (Jordan, 1982), *Mesorhizobium* (Jarvis *et al*., 1997), *Rhizobium* (Jordan, 1984) and *Sinorhizobium* (Chen *et al*., 1988; de Lajudie *et al*., 1994) are symbiotic nitrogen-fixers that form root and/or stem nodules on various leguminous plants. The taxonomy of these bacteria is currently under revision, and novel species are regularly being proposed (Amarger *et al*., 1997; van Berkum *et al*., 1998; Nick *et al*., 1999; Wang *et al*., 1998, 1999a). Close relationships between the rhizobia and non-nodulating bacteria have been reported, for example between *Agrobacterium rhizogenes* and *Rhizobium tropici*.

Using PCR-amplified 16S rRNA gene RFLP, multilocus enzyme electrophoresis (MLEE), cellular plasmid electrophoresis and *nifH* and *nodDAB* Southern hybridization, we have identified several genomic groups (rDNA types) among the rhizobial isolates associated with *Leucaena leucocephala* in Mexican soils (Wang *et al*., 1999b). One of these groups, rDNA type 11, was closely related to *Rhizobium giardinii* based on the analysis of RFLP patterns of PCR-amplified 16S rRNA genes. Isolates of rDNA type 11 formed a minor proportion of the rhizobia from about 5% of the nodules of *L. leucocephala*. Since *R. giardinii* (Amarger *et al*., 1997) represents a phylogenetic branch that is distantly related to other *Rhizobium* species, we were interested in these *R. giardinii*-related bacteria. To verify the taxonomic and phylogenetic positions of this novel group, we characterized the isolates within the rDNA type 11 group further using 16S rRNA gene sequencing, DNA–DNA hybridization and phenotypic analysis. In this study, the phylogenetic relationships between rDNA type 11, *Sinorhizobium* species and *Ensifer adhaerens* were found to be closer than those between this novel group and *R. giardinii*. The DNA relatedness obtained from DNA–DNA hybridization and the phenotypic characterization indicated

Abbreviation: MLEE, multi-locus enzyme electrophoresis.

The GenBank accession number for the 16S rRNA gene sequence of strain Lc04^T is AY024335.

that the novel group was distinct from all *Sinorhizobium* species, *E. adhaerens* and *R. giardinii* and we therefore propose a novel species within the genus *Sinorhizobium*, *Sinorhizobium morelense* sp. nov., for this group.

METHODS

Isolates and strains. The bacteria used in this research are listed in Table 1. Since the isolates within rDNA type 11 were fast-growing, acid-producing rhizobia (Wang *et al*., 1999b), type strains of species within the genera *Agrobacterium*, *Rhizobium* and *Sinorhizobium* were included as references. All strains were kept in PY medium (peptone of casein, 5·0 g; yeast extract, 3·0 g; $CaCl_2$, 0·6 g; distilled water, 1 l) at 4 °C for temporary storage and in 20 % glycerol at −20 °C for long-term storage.

Sequencing and phylogenetic analysis of 16S rRNA genes. Isolate Lc04^T, representative of rDNA type 11, was used for 16S rRNA gene sequencing using the method of direct sequencing from PCR products (Hurek *et al*., 1997). The sequence obtained was deposited in the GenBank database and was compared with related sequences from the database. The sequences were aligned using the PILEUP program in the Wisconsin package version 8.1 (Genetics Computer Group, 1995). A phylogenetic tree was reconstructed and bootstrapped with 1000 replications of each sequence using CLUSTAL W (Thompson *et al*., 1994). The tree was visualized by using the program TreeView (Page, 1996).

DNA–DNA hybridization and DNA base composition. DNA extraction and Southern hybridization were performed as described previously (Wang *et al*., 1998) to estimate the DNA–DNA relatedness among isolates of rDNA type 11 and the reference strains. For DNA–DNA hybridizations between strain Lc04^T and *E. adhaerens* strains, a microplate method modified from Ezaki *et al*. (1989) as described by Willems *et al*. (2001) was used, with hybridizations carried out at 45 °C. For determination of DNA base composition, DNA of strain Lc04^T was degraded enzymically into nucleosides as described by Mesbah *et al*. (1989). The resulting nucleoside mixtures were separated by HPLC using a Waters Symmetry Shield C8 column at 37 °C. The solvent was 0·02 M $NH_4H_2PO_4$ (pH 4·0) with 1·5 % acetonitrile. Non-methylated lambda phage DNA (Sigma) was used as the calibration reference.

Phenotypic characterization. Methods described previously (Wang *et al*., 1998) were used to analyse the utilization of sole carbon and nitrogen sources, resistance to antibiotics, tolerance of 0·5–4·0 % (w/v) NaCl, the pH and temperature ranges for growth and growth in LB medium and in litmus milk. Anaerobic growth by reduction of nitrate or sulfate was determined in YM broth supplemented with 6 mM K_2NO_3 or Na_2SO_4 (Daniel *et al*., 1982). Gram staining and cellular morphology were examined using routine methods.

Nodulation tests. Cross-nodulation was performed using strains Lc04^T, Lc18, Lc56a and Lc57 to inoculate *L. leucocephala*, *Phaseolus vulgaris* and *Acacia farnesiana*, as described by Vincent (1970).

RESULTS AND DISCUSSION

Sequencing and phylogeny of 16S rRNA genes

The 16S rRNA gene sequence obtained from Lc04^T was 1436 bp long. According to the sequence analysis, isolate Lc04^T was most closely related to *Sinorhizobium* species. It had around 98 % sequence identity to *Sinorhizobium fredii*, *Sinorhizobium xinjiangense*, *Sinorhizobium meliloti* and *Sinorhizobium medicae* and 97 % sequence identity to the other *Sinorhizobium* species. *E. adhaerens*, a non-symbiotic soil bacterium (Casida, 1982), showed 98 % sequence identity to isolate Lc04^T. The sequence identity between *Mesorhizobium* species and isolate Lc04^T ranged from 94 to

Table 1. Rhizobial isolates and strains used in this study

Isolate or strain	Host plant	Geographical origin	Reference
rDNA type 11 (*Sinorhizobium morelense* sp. nov.) isolates Lc04^T, Lc18, Lc19, Lc21, Lc29, Lc44, Lc56a	*L. leucocephala*	Mexico	Wang *et al*. (1999b)
Other rDNA type 11 isolate Lc57	*L. leucocephala*	Mexico	Wang *et al*. (1999b)
Agrobacterium tumefaciens HAMBI 1811^T			
Agrobacterium vitis HAMBI 1817^T			
Ensifer adhaerens ATCC 33212^T, ATCC 33499			Balkwill (2002)
Rhizobium gallicum R602sp^T	*Phaseolus vulgaris*	France	Amarger *et al*. (1997)
Rhizobium giardinii H152^T	*P. vulgaris*	France	Amarger *et al*. (1997)
Rhizobium leguminosarum USDA 2370^T	*Pisum sativum*	USA	Jordan (1984)
Rhizobium mongolense USDA 1844^T	*Medicago* sp.	China	van Berkum *et al*. (1998)
Rhizobium tropici CIAT 899^T	*L. leucocephala*	Colombia	Martínez-Romero *et al*. (1991)
Sinorhizobium arboris			
HAMBI 1552^T	*Prosopis chilensis*	Sudan	Nick *et al*. (1999)
HAMBI 1396	*P. chilensis*	Kenya	Nick *et al*. (1999)
HAMBI 1700, HAMBI 1624	*Acacia senegal*	Sudan	Nick *et al*. (1999)
Sinorhizobium kostiense HAMBI 1489^T, HAMBI 1501	*A. senegal*	Sudan	Nick *et al*. (1999)
Sinorhizobium fredii USDA 205^T, USDA 202, USDA 191	*Glycine max*	China	Chen *et al*. (1988)
Sinorhizobium medicae			
A320^T	*Medicago* sp.		Rome *et al*. (1996)
m75			Eardly *et al*. (1990)
Sinorhizobium meliloti			
USDA 1002^T	*Medicago sativa*		Jordan (1984)
74B12			Eardly *et al*. (1990)
Sinorhizobium saheli LMG 77837^T, LMG 8310, LMG 11864	*Sesbania* sp.	Senegal	de Lajudie *et al*. (1994)
Sinorhizobium terangae LMG 7834^T	*Acacia laeta*	Senegal	de Lajudie *et al*. (1994)
Sinorhizobium xinjiangense CCBAU 110^T, CCBAU 105, Rx41	*G. max*	China	Chen *et al*. (1988)

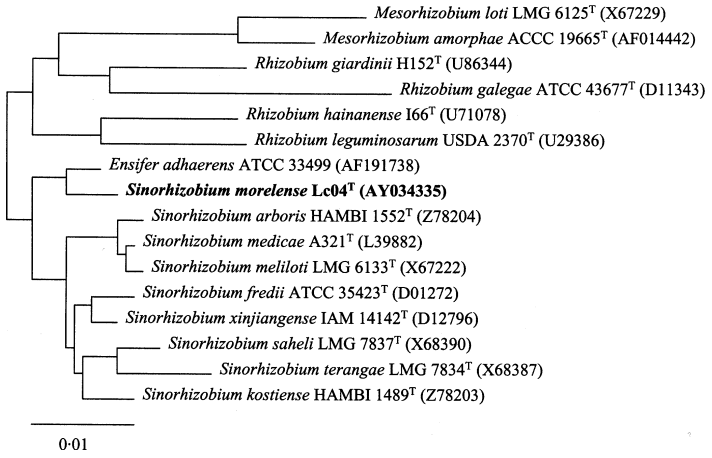

Fig. 1. Simplified phylogram of 16S rRNA gene sequences obtained in this research and in GenBank showing the phylogenetic position of rDNA type 11, represented by isolate Lc04^T. Sequences were aligned using the PILEUP program in the GCG package (Genetics Computer Group, 1995). CLUSTAL W (Thompson *et al.*, 1994) was used to reconstruct the tree and bootstrap the tree from 1000 replications of the aligned sequences. Accession numbers of the nucleotide sequences are indicated in parentheses. Bar, 1% nucleotide substitution.

Table 2. DNA relatedness between isolate Lc04^T and other isolates of rDNA type 11 and reference strains

Values were obtained by Southern hybridization of total DNA unless indicated.

Isolate or strain	DNA relatedness (%) to isolate Lc04^T
rDNA type11	
Lc04^T	100
Lc18	80·2 ± 2·4
Lc57	79·8
Lc56a	99·2
E. adhaerens ATCC 33212^T	41*
E. adhaerens ATCC 33499	39*
S. arboris LMG 1489^T	33·6 ± 12·0
S. kostiense LMG 1552^T	34·5 ± 3·2
S. fredii USDA 205^T	19·3
S. medicae USDA 1037^T	39·0 ± 0·5
S. meliloti USDA 1002^T	17·5 ± 1·9
S. saheli LMG 7837^T	31·4 ± 0·6
S. terangae LMG 7834^T	31·4 ± 0·6
S. xinjiangense CCBAU 110^T	27·5 ± 4·8
Agrobacterium tumefaciens HAMBI 1811^T	10·8 ± 1·0
Agrobacterium vitis HAMBI 1817^T	20·8
R. galegae USDA 4128^T	29·2 ± 8·2
R. gallicum R603sp^T	12·0 ± 1·1
R. giardinii H152^T	21·5 ± 3·9
R. leguminosarum USDA 2370^T	17·2
R. mongolense USDA 1844^T	18·3
R. tropici CIAT 899^T	18·9 ± 4·8

* Value obtained using a microplate hybridization method.

95%. The sequence identity was 96% between isolate Lc04^T and *R. giardinii* and it was lower for other *Rhizobium* species. Isolate Lc04^T clustered with *E. adhaerens* in the reconstructed phylogenetic tree (Fig. 1). These two species formed the most divergent branch within the *Sinorhizobium* group. The phylogenetic relationships among the defined rhizobial species were similar in our reconstructed tree and previously published trees (e.g. de Lajudie *et al.*, 1998a, b; Wang *et al.*, 1999a). In contrast to the relationship estimated from 16S rRNA gene RFLP analysis (Wang *et al.*, 1999b), isolates of rDNA type 11 were found to belong to the *Sinorhizobium* phylogenetic lineage on the basis of comparative analysis of 16S rRNA gene sequences (Fig. 1). The different relationships revealed previously by PCR-RFLP and by sequencing analyses might indicate a limitation of PCR-RFLP analysis in accurate estimation of phylogenetic relationships. In Fig. 1, the close relationship between *E. adhaerens*, *Sinorhizobium* species and isolate Lc04^T offered further evidence that the symbiotic species may have a common origin with non-symbiotic bacteria (Wang & Martínez-Romero, 2000).

DNA G+C content and DNA–DNA hybridization

The DNA G+C content of strain Lc04^T was 61·7 mol%. The data for DNA–DNA relatedness obtained from hybridization are presented in Table 2. The four representative isolates of rDNA type 11 had DNA relatedness of 79·8–100%. The values of DNA relatedness were 39–41% between the group 11 isolate Lc04^T and *Ensifer* strains, 17·5–39·0% between isolate Lc04^T and type strains of *Sinorhizobium* species and 10·8–29·2% between isolate Lc04^T and the type strains of some *Agrobacterium* and *Rhizobium* species. Although the use of 70% DNA relatedness as a threshold for bacterial species has been seriously criticized (Ward, 1998), this criterion has been used in the description of many rhizobial species, such as *Mesorhizobium ciceri* (Nour *et al.*, 1994) and *Rhizobium hainanense* (Chen *et al.*, 1997). In this study, the large gap between the relatedness among rDNA type 11 isolates and that between this group and other species indicated that the isolates of rDNA type 11 represent a unique genomic species, different from the described species of the genera *Ensifer*, *Rhizobium* and *Sinorhizobium*. This conclusion was also supported by MLEE results reported previously (Wang *et al.*, 1999b).

Phenotypic characterization

A total of 113 phenotypic features was analysed for the isolates and reference strains. None of bacteria tested could use 2-anthranilic acid, benzoic acid, coumaric acid, fluorobenzoic acid, propionic acid or salicylic acid as sole carbon sources and all were sensitive to 1 mg kanamycin ml^{-1} and 500 μg neomycin ml^{-1}. None could grow anaerobically in YM broth with or without sodium sulfate (6 mM). All eight isolates could grow anaerobically in YM broth by denitrification. Isolate Lc57 was different in many aspects from the other seven isolates. The seven isolates were able to use most of the compounds tested, including sugars, organic acids, amino acids and alcohols, as sole carbon sources. However, they grew better and formed larger colonies with di- or trisaccharides (sucrose, lactose, maltose, melibiose, raffinose, trehalose or melezitose), organic acids and amino acids than with monosaccharides (such as fructose, glucose, fucose, xylose, glycerol or mannitol). They could use most of the amino acids, except DL-alanine and urea, as sole sources of nitrogen. Growth was improved by adding biotin as a growth factor in defined media and they grew in defined medium supplemented with hydrolytic casein as the sole carbon and nitrogen source. The seven isolates were resistant to (μl^{-1}) 1 mg carbenicillin, 500 μg kanamycin, 300 μg erythromycin, 100 μg neomycin and streptomycin and 5 μg gentamicin and chloramphenicol, but sensitive to 5 μg tetracycline and 20 μg nalidixic acid ml^{-1}. They could grow at pH 5·0–10·0 and were tolerant of 2·0 % (w/v) NaCl. They could not grow at 37 °C. The generation time ranged from 1·5 to 2·0 h. Multiplication by budding was observed in isolates Lc04^T and Lc18. These results implied that the seven isolates of rDNA type 11 were a group phenotypically different from other species. The distinctive features of rDNA type 11, *E. adhaerens*, *Sinorhizobium* species and *R. giardinii* are listed in Table 3.

Nodulation tests

Strains Lc04^T, Lc18 and Lc56a were incapable of forming nodules on *L. leucocephala*, *A. farnesiana* or on *P. vulgaris*, while nodules were obtained on *L. leucocephala* plants inoculated with isolate Lc57. Bacteria isolated from the root nodules of some legume plants have been shown to be closely related to or to be member of the non-symbiotic genus *Agrobacterium* (Tan *et al.*, 1999). Therefore, we postulate that the non-nodulating isolates in rDNA type 11 may, as has been reported for *Agrobacterium* strains, have simply entered the nodules opportunistically. Alternatively, the bacteria may have lost their symbiotic plasmid after isolation from nodules.

Based on all the data presented in this work and in our previous report (Wang *et al.*, 1999b), it is clear that the non-nodulating strains in rDNA type 11 are a group related to the genera *Sinorhizobium* and *Ensifer*. Since this group is phylogenetically, genetically and phenotypically distinct from the eight species within the genus *Sinorhizobium*, *S. meliloti*, *Sinorhizobium saheli* (de Lajudie *et al.*, 1994), *Sinorhizobium terangae* (de Lajudie *et al.*, 1994), *S. medicae* (Rome *et al.*, 1996), *Sinorhizobium fredii* (Chen *et al.*, 1988), *S. xinjiangense* (Chen *et al.*, 1988), *Sinorhizobium arboris* (Nick *et al.*, 1999) and *Sinorhizobium kostiense* (Nick *et al.*, 1999), and different from *Ensifer adhaerens* (Casida, 1982), we conclude that rDNA type 11 is a novel species.

E. adhaerens is a Gram-negative soil bacterium that adheres to other bacteria and may cause their lysis (Casida, 1982). The 16S rRNA gene sequence of *E. adhaerens* revealed that it was related to *Sinorhizobium*, although it was considered to be outside this genus (Balkwill, 2002). We have shown elsewhere that *E. adhaerens* constitutes a group of non-nodulating bacteria that do not harbour *nifH* genes. Furthermore, it has been reported that *E. adhaerens* ATCC 33499 could be converted into a nitrogen-fixing symbiont by the introduction of the symbiotic plasmid from *R. tropici* to this bacterium (Rogel *et al.*, 2001). The results of our own work indicate that there are both symbiotic and non-symbiotic strains in rDNA type 11, as reported for *Rhizobium etli* (Segovia *et al.*, 1991). Collectively, all these data imply that *Ensifer* and *Sinorhizobium* could be seen as synonyms. The older name (*Ensifer*) would have priority over the younger one (*Sinorhizobium*). However, the community of users of rhizobial taxonomy will not welcome a change of *Sinorhizobium* to *Ensifer* and we therefore propose to describe rDNA group 11 as a novel species in *Sinorhizobium*. This synonymy of *Sinorhizobium* and *Ensifer* will be discussed in detail in a future manuscript.

In our previous work (Wang *et al.*, 1999b), strain Lc57 was identified as type 11 since it had a 16S rRNA gene PCR-RFLP pattern identical to the other seven strains. In MLEE analysis, this strain had an electrophoretic type different from, but very similar to, that of the other seven strains (Wang *et al.*, 1999b). However, strain Lc57 was quite different from the other seven strains in its phenotypic characters. It was sensitive to antibiotics that the other seven strains were resistant to. It had a slower growth rate. Colonies were $\leqslant$ 1 mm after 5 days incubation on PY medium and had less exopolysaccharide than those of the other seven isolates. Strains Lc57 and Lc04^T had 79·8 % DNA–DNA relatedness. We hesitated to include strain Lc57 in the same species as the other seven strains in view of these documented differences. Nevertheless, the close relationship between strain Lc57 and the non-nodulating strains in rDNA type 11 is further evidence that rhizobia may have a common ancestry with other bacteria.

Taking into consideration the data from our previous report (Wang *et al.*, 1999b) and from this study, as well as the suggestions of Graham *et al.* (1991) and Wayne *et al.* (1987), we propose a novel species, *Sinorhizobium morelense* sp. nov., for the seven strains of rDNA type 11 from *L. leucocephala*. This is the fifth rhizobial species that is from leguminous trees in tropic regions.

Table 3. Distinctive features of strains of rDNA type 11 and related species

Species are identified as: 1, *E. adhaerens*; 2, *R. giardinii*; 3, *S. meliloti*; 4, *S. medicae*; 5, *S. fredii*; 6, *S. xinjiangense*; 7, *S. saheli*; 8, *S. terangae*; 9, *S. arboris*; 10, *S. kostiense*. d, Variable; ND, no data available.

Characteristic	rDNA type 11		1	2	3	4	5	6	7	8	9	10
	7 strains	Lc57										
Signature sequence in 16S rRNA*	−	ND	+	−	−	−	−	−	−	−	−	−
Division by budding	+	−	+	−	−	−	−	−	−	−	−	−
Growth in LB	+	−	ND	−	+	+	d	−	−	+	+	−
Tolerance of:												
2% NaCl	+	−	+	+	+	+	d	−	−	+	+	−
2·5% NaCl	−	−	+	+	d	d	d	−	+	−	−	+
Growth at:												
37 °C	−	+	+	−	+	+	+	d	+	+	+	+
pH 5·0	+	−	ND	+	−	+	d	−	−	−	−	−
pH 10·0	+	−	ND	−	−	+	−	−	−	−	−	−
Alkali production in litmus milk	−	+	ND	+	+	+	+	+	+	+	+	+
Anaerobic growth by denitrification	+	+	+	−	d	−	−	+	−	+	d	−
Utilization of sole carbon sources:												
DL-Arabinose	−	+	ND	+	+	+	d	d	+	−	d	+
Dulcitol	−	−	ND	+	d	−	−	−	−	−	+	−
Inositol	−	+	ND	−	+	+	d	+	+	−	+	+
Citrate	+	−	ND	+	−	−	d	−	−	−	−	−
Glycine	+	−	ND	−	−	−	−	−	−	−	+	−
L-Tryptophan	+	−	ND	−	−	−	−	−	−	−	+	−
Acetate	+	−	+	−	−	−	d	−	+	−	−	−
Arabitol	−	−	ND	+	+	+	d	d	−	+	+	+
Resistance to antibiotics (μg ml^{-1}):												
Carbenicillin (1000)	+	−	ND	−	−	−	−	−	−	−	−	−
Kanamycin (500)	+	−	ND	−	−	−	−	−	−	−	−	−
Erythromycin (300)	+	−	ND	−	−	−	−	−	−	−	−	−
Streptomycin (100)	+	−	ND	−	−	−	−	−	−	−	−	−
Neomycin (100)	+	−	ND	−	−	−	−	−	−	−	−	−
Gentamicin (5)	+	−	ND	−	+	+	d	−	−	+	+	d
Nalidixic acid (20)	−	+	ND	−	−	+	d	+	+	+	+	+

* Signature sequence is TACGGAGACGTTT.

The others are *R. tropici* (Martínez-Romero *et al.*, 1991), *S. terangae* (de Lajudie *et al.*, 1994), *S. arboris* and *S. kostiense* (Nick *et al.*, 1999). The novel species can be differentiated from *E. adhaerens* and related rhizobial species as shown in Table 3. The poor growth of the isolates on monosaccharides in comparison with di- or trisaccharides and organic acids as sole carbon sources might reflect the adaptation of the isolates to the soil and rhizosphere. Antibiotic resistance in clinical bacteria has been pointed out as a serious problem and the origin and lateral transfer of antibiotic-resistance genes have been widely studied (see reviews by Nikaido, 1998; Rowe-Magnus & Mazel, 1999), as have the mechanisms of multiple antibiotic resistance and efflux (Nikaido, 1998). The biological basis of multiple antibiotic resistance in group 11 isolates is worth investigating, since these isolates originate from sites with no known history of antibiotic use.

Description of *Sinorhizobium morelense* sp. nov.

Sinorhizobium morelense (mo.re.len′se. N.L. neut. adj. *morelense* of Morelos, the name of a state in Mexico, where the bacterium was isolated).

Gram-negative, non-spore-forming rods. Multiply by budding. Aerobic, but can grow anaerobically by denitrification. Generation time in PY broth is 1·5–2·0 h at 28 °C. Growth is inhibited on PY medium at 37 °C. Colonies on PY or YMA are circular, cream-coloured, semi-translucent and mucilaginous. Colonies are normally more than 2 mm in diameter within 3 days. A wide range of carbohydrates and amino acids is utilized as sole carbon sources for growth. However, grows less well on monosaccharides than on di- or trisaccharides. Most amino acids, except DL-alanine and urea, can be used as sole nitrogen sources for growth. Addition of biotin in defined medium can improve growth. Growth is obtained at pH 5·0–10·0.

The most distinctive feature is multiple antibiotic resistance. The seven strains can grow in PY medium supplied with 1 mg carbenicillin, 500 μg kanamycin, 300 μg erythromycin, 100 μg neomycin or streptomycin or 5 μg chloramphenicol or gentamicin ml^{-1}. Sensitive to 5 μg tetracycline and 20 μg nalidixic acid ml^{-1}. In litmus milk, most strains do not produce alkali and the final pH is 7·0–7·5 after 4 weeks incubation.

The type strain is Lc04^T (= CFN E1007^T = LMG 21331^T). The G+C content of this strain is 61·7 mol %. This strain has all the distinctive and descriptive features of the species. Its generation time in PY is 1·5 h at 28 °C. No symbiotic plasmid is detected.

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